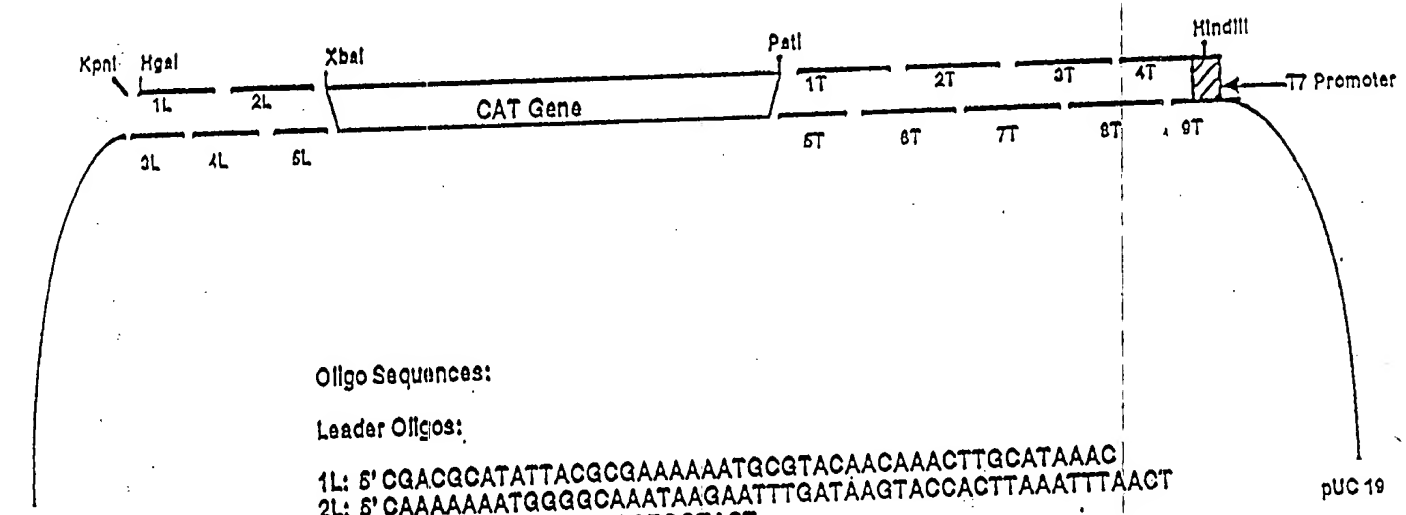


Oligo Sequences:

Leader Oligos:

1L: 5' CGACGCATATTACGCGAAAAAATGCGTACAACAAACTTGCATAAAC
2L: 5' CAAAAAAATGGGGCAAATAAGAATTTGATAAGTACCACTTAAATTTAACT
3L: 5' CTAGAGTTAAATTTAAGTGGTACT
4L: 5' TATCAAATTCTTATTTGCCCCATTTTTTTGGTTTATGCAAGTTTGTTGTA
5L: 5' CGCATTTTTTCGCGTAATATGCGTCGGTAC

Trailer Oligos:

1T: 5' GTATTCAATTATAGTTATTAAAAATTAAAAATCATATAATTTTTTAAATA
2T: 5' ACTTTTAGTGAACTAATCCTAAAGTTATCATTTTAATCTTGGAGGAATAA
3T: 5' ATTTAAACCCTAATCTAATTGGTTTATATGTGTATTAACTAAATTACGAG
4T: 5' ATATTAGTTTTTGACACTTTTTTTCTCGTTATAGTGAGTCGTATTA
5T: 5' AGCTTAATACGACTCACTATAACGA
6T: 5' GAAAAAAAGTGTCAAAAACTAATATCTCGTAATTTAGTTAATACACATAT
7T: 5' AAACCAATTAGATTAGGGTTTAAATTTATTCCTCCAAGATTAAAATGATA
8T: 5' ACTTTAGGATTAGTTCACTAAAAGTTATTTAAAAAATTATATGATTTTTA
9T: 5' AATTTTAATAACTATAATTGAATACTGCA

FIG. 1

7682-052 (Sheet 1 of 28)

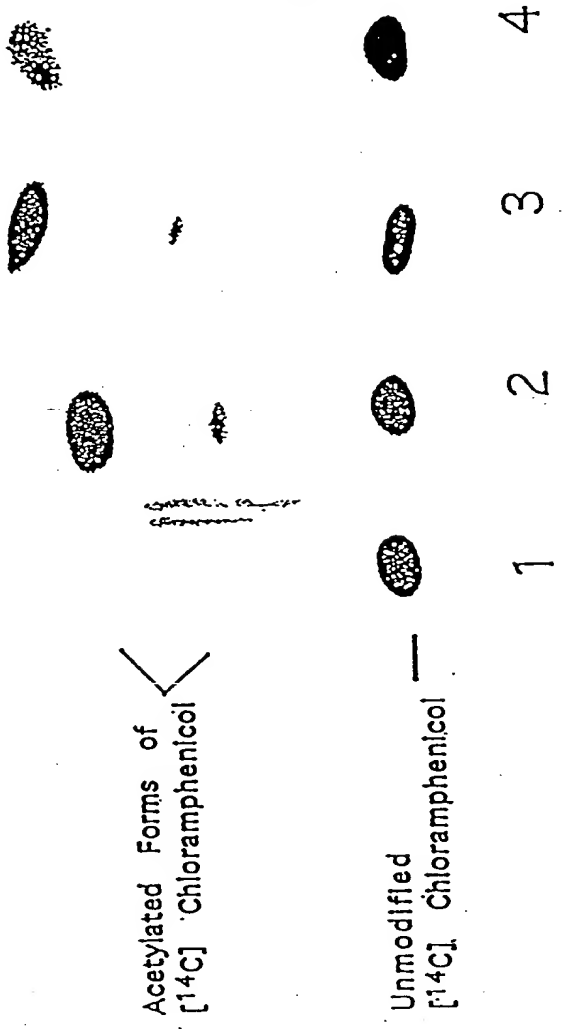
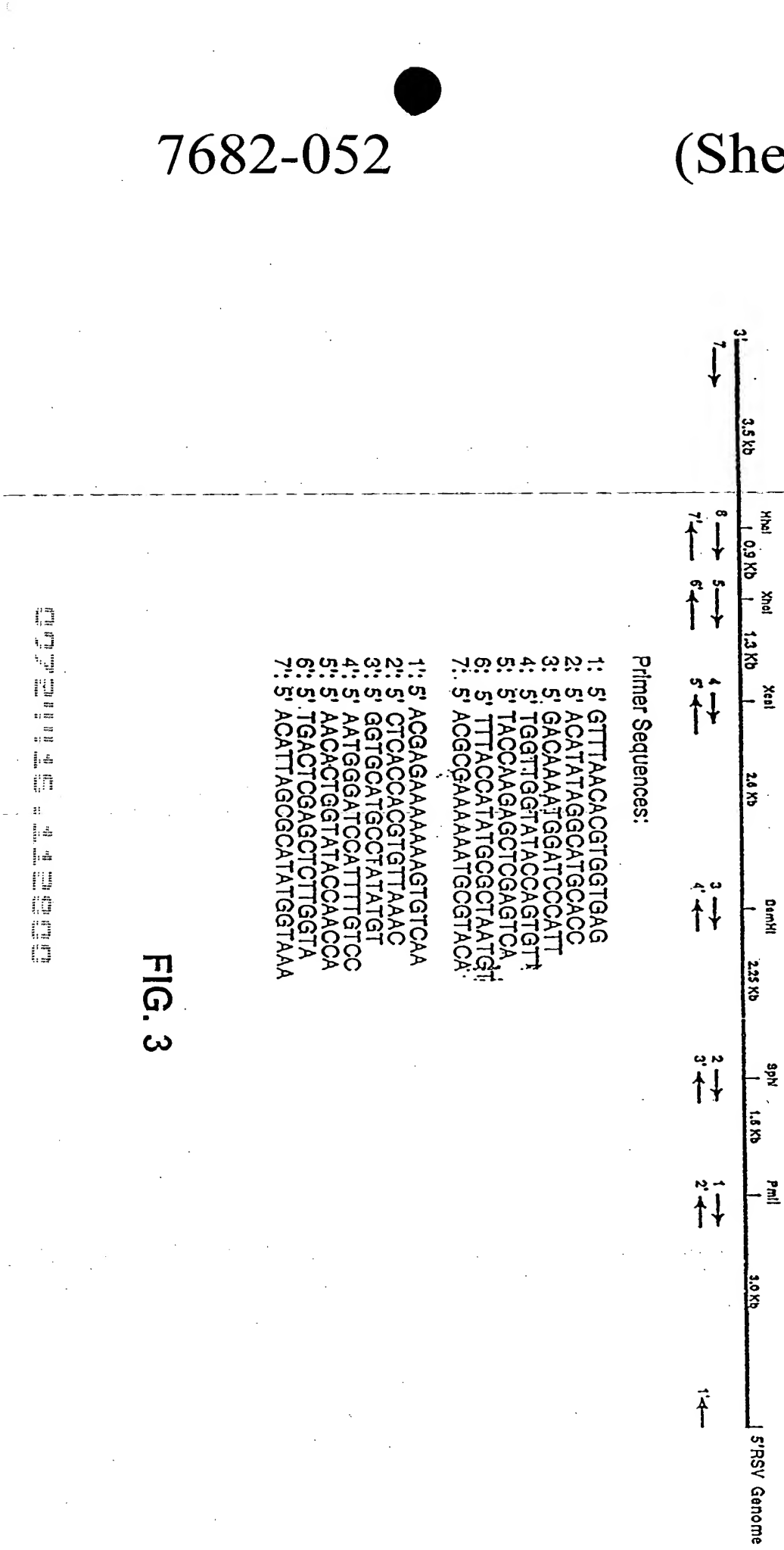

4
3
2
1
Acetylated Forms of [14C] Chloramphenicol
Unmodified [14C] Chloramphenicol

FIG. 2

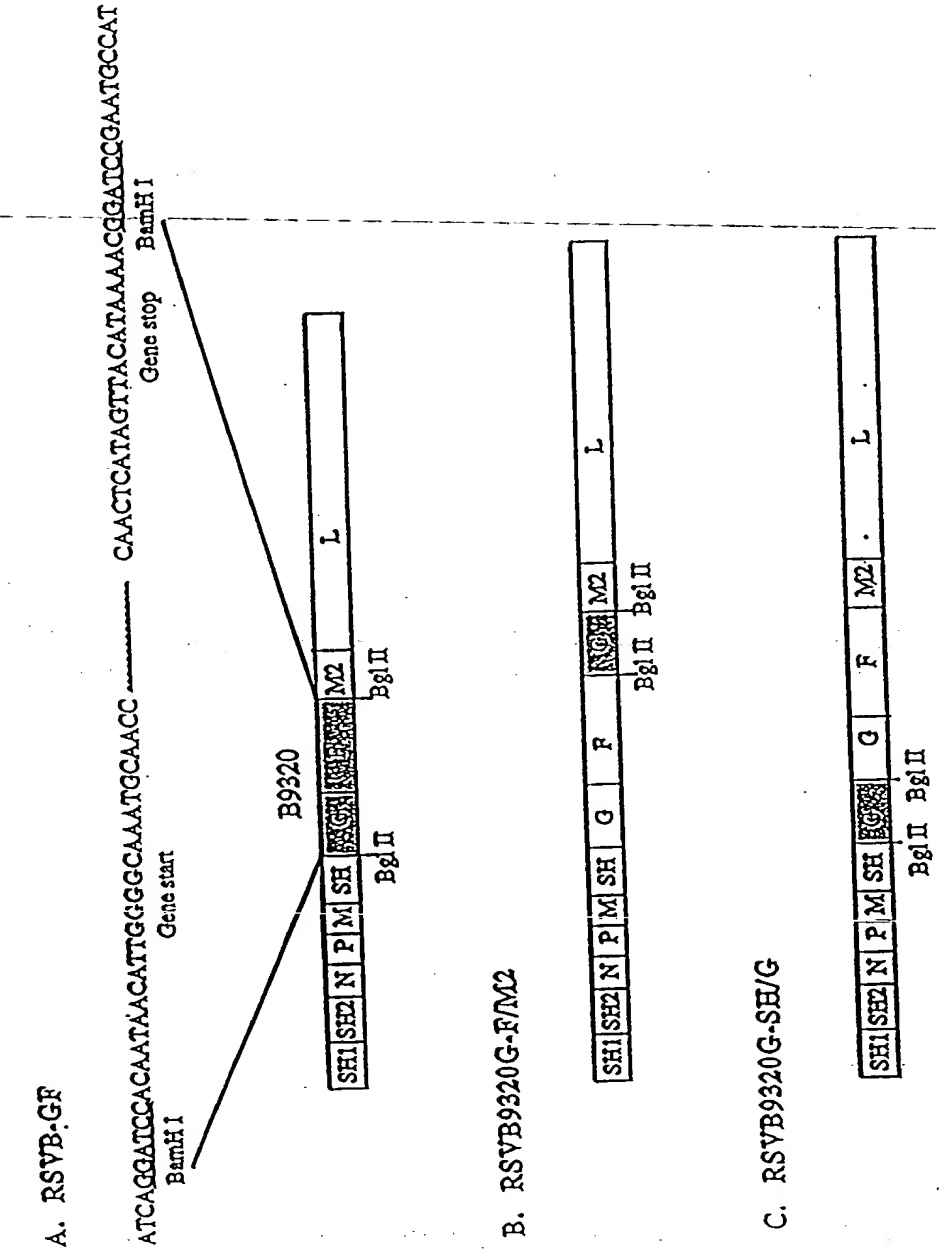


Primer Sequences:

1: 5' GTTTAACACGTGGTGAG
2: 5' ACATATAGGCATGCACC
3: 5' GACAAAATGGATCCCATT
4: 5' TGGTTGGTATACCAGTGTT
5: 5' TACCAAGAGCTCGAGTCA
6: 5' TTTACCATATGCGCTAATGT
7: 5' ACGCGAAAAAATGCGTACA

1': 5' ACGAGAAAAAAAGTGTCAA
2': 5' CTCACCACGTGTTAAAC
3': 5' GGTGCATGCCTATATGT
4': 5' AATGGGATCCATTTTGTCC
5': 5' AACACTGGTATACCAACCA
6': 5' TGACTCGAGCTCTTGGTA
7': 5' ACATTAGCGCATATGGTAAA

FIG. 3


A. RSVB-GF
ATCAGGATCCACAATAACATTGGGGCAAATGCAACC
BamH I
Gene start
CAACTCATAGTTACATAAAACGGATCCGAATGCCAT
Gene stop
BamH I
B9320
SH1 SH2 N P M SH G F M2 L
Bgl II
Bgl II
B. RSVB9320G-F/M2
SH1 SH2 N P M SH G F M2 L
Bgl II Bgl II
C. RSVB9320G-SH/G
SH1 SH2 N P M SH G F M2 L
Bgl II Bgl II

FIGS. 4A-C

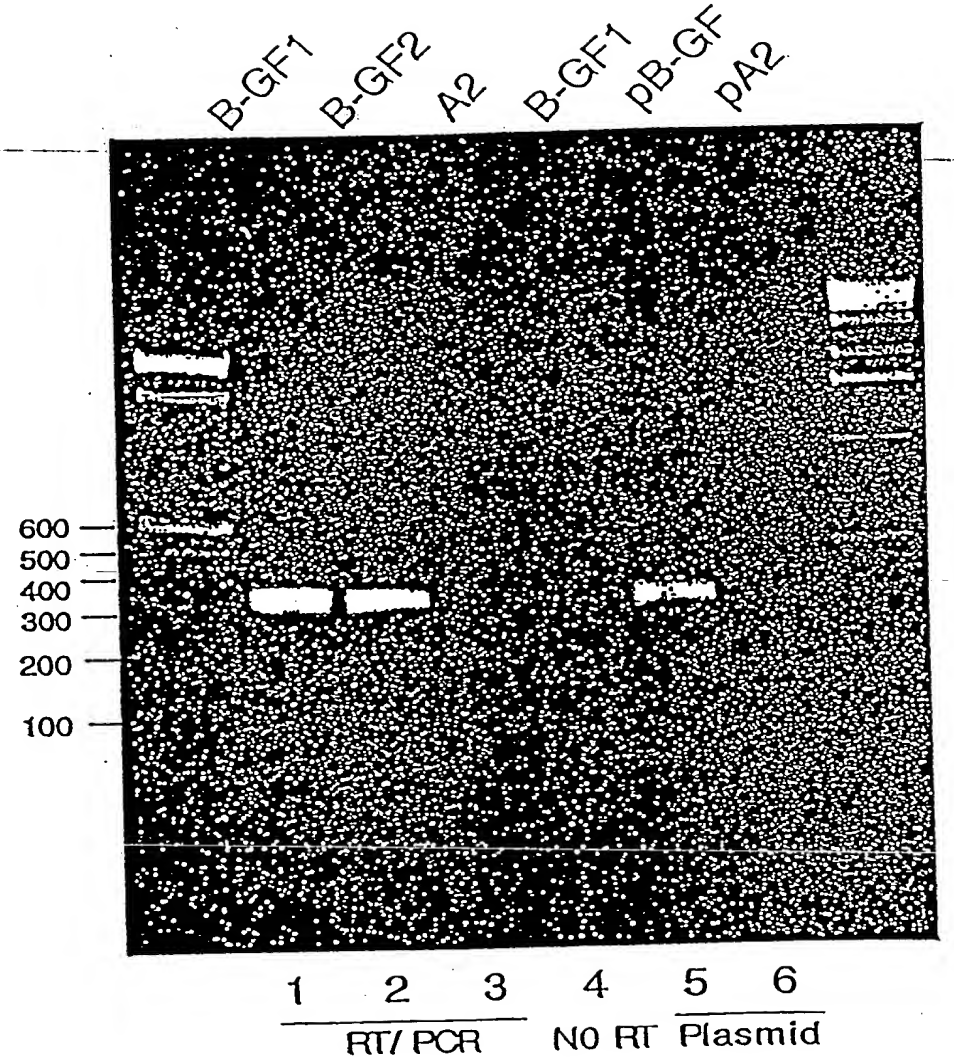
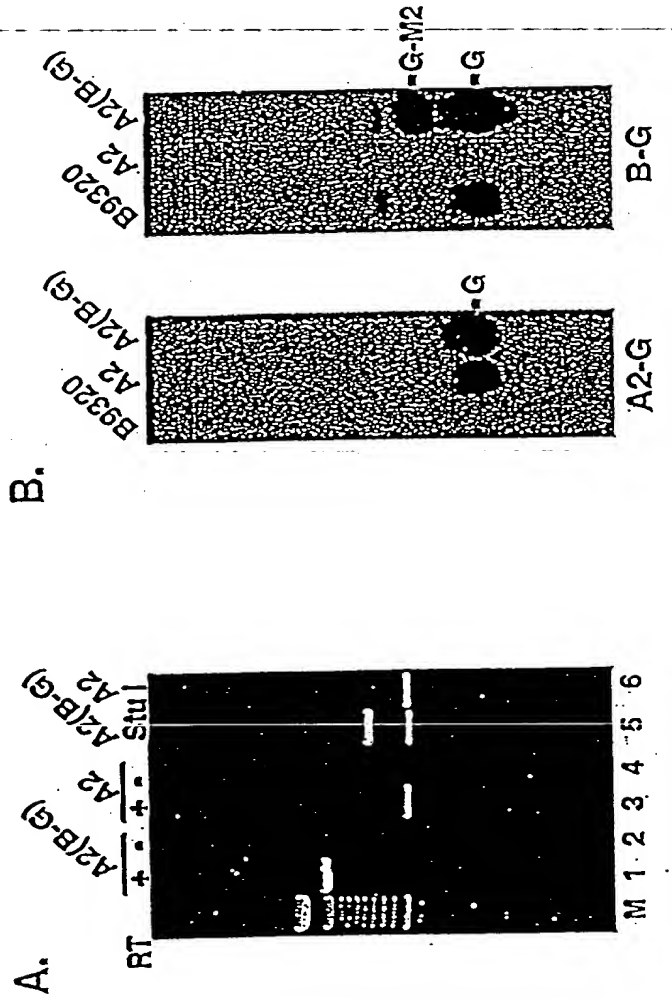

B-GF1
B-GF2
A2
B-GF1
pB-GF
pA2
600
500
400
300
200
100
1
2
3
4
5
6
RT/ PCR
NO RT
Plasmid

FIG. 5


A.
RT
A2/B-G
A2
A2/B-G
Stu I
A2
M 1 2 3 4 5 6
B.
B-G
B9320
A2
A2/B-G
G-M2
G
A2-G
B9320
A2
A2/B-G
G

FIGS. 6A-B

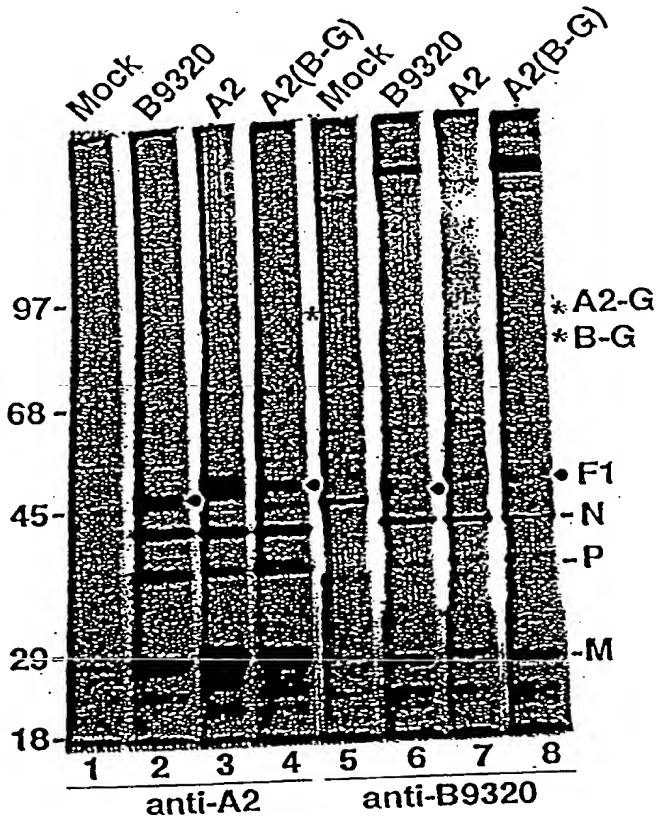

Mock
B9320
A2
A2(B-G)
Mock
B9320
A2
A2(B-G)
97-
68-
45-
29-
18-
*A2-G
*B-G
•F1
-N
-P
-M
1 2 3 4 5 6 7 8
anti-A2
anti-B9320

FIG. 7

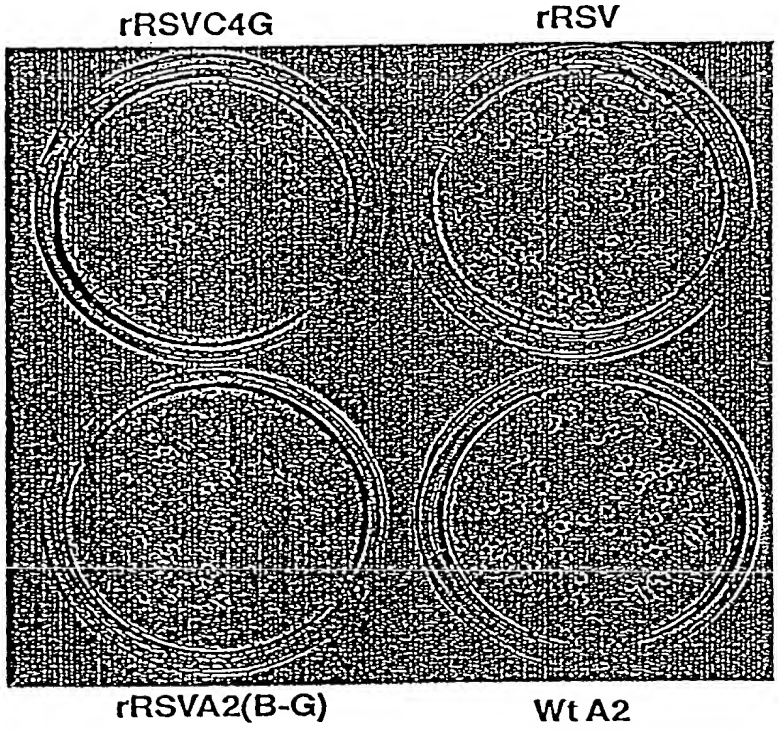


FIG. 8

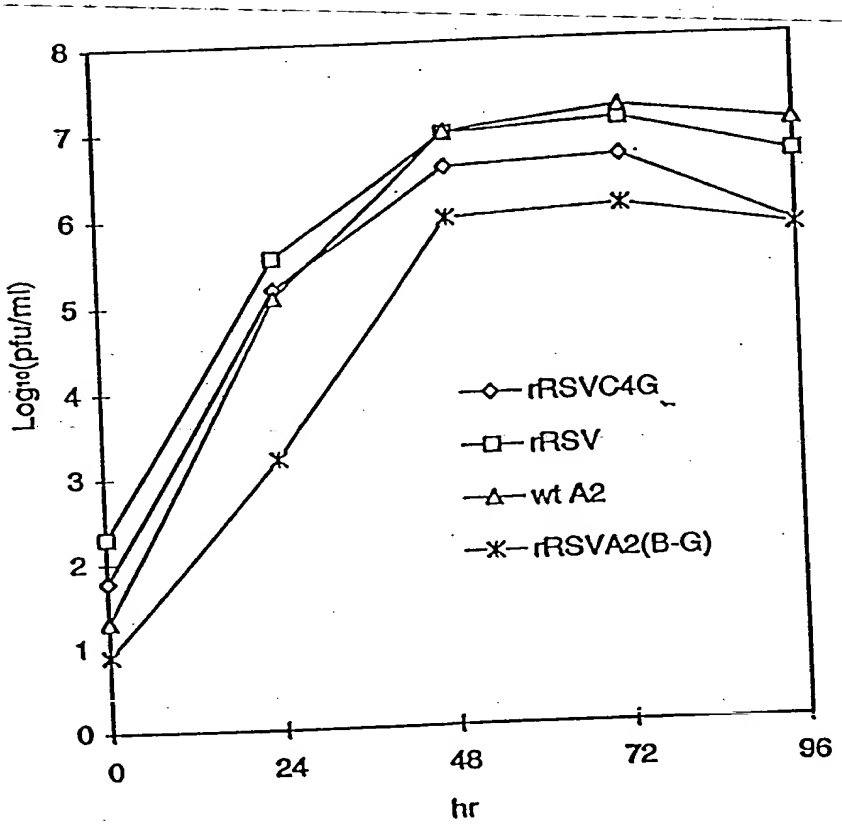

8
7
6
5
4
3
2
1
0
Log10(pfu/ml)
0
24
48
72
96
hr
rRSVC4G
rRSV
wt A2
rRSVA2(B-G)

FIG. 9

```
MDPIINGNSANVYLT DSYLKGVISFSECNA LGSYIFNGPYLKNDY TNLISRQNPLIEHMN LKKLNITQSLISKYH 75
KGEIKLEEPTYFQSL LMTYKSMTSSEQIAT TNLLKKIIRRAIEIS DVKVYAILNKLGLKE KDKIKSNNGQDEDNS 150
VITTIIKDDILSAVK DNQSHLKADKNHSTK QKDTIKTTLLKKLMC SMQHPPSWLIHWFNL YTKLNNILTQYRSNE 225
VKNHGFTLIDNQTLS GFQFILNQYGCIVYH KELKRITVTTYNQFL TWKDISLSRLNVCLI TWISNCLNTLNKSLG 300
LRCGFNNVILTQLFL YGDCILKLFHNEGFY IIKEVEGFIMSLILN ITEEDQFRKRFYNSM LNNITDAANKAQKNL 375
LSRVCHTLLDKTVSD NIINGRWIILLSKFL KLIKLAGDNNLNNLS ELYFLFRIFGHPMVD ERQAMDAVKINCNET 450
KFYLLSSLSMLRGAF IYRIIKGFVNNYNRW PTLRNAIVLPLRWLT YYKLNTYPSLLELTE RDLIVLSGLRFYREF 525
RLPKKVDLEMIINDK AISPPKNLIWTSFPR NYMPSHIQNYIEHEK LKFSESDKSRRVLEY YLRDNKFNECDLYNC 600
VVNQSYLNNPNHVVS LTGKERELSVGRMFA MQPGMFRQVQILAEK MIAENILQFFPESLT RYGDLELQKILELKA 675
GISNKSNRYNDNYNN YISKCSIITDLSKFN QAFRYETSCICSDVL DELHGVQSLFSWLHL TIPHVTIICTYRHAP 750
PYIGDHIVDLNNVDE QSGLYRYHMGGIEGW CQKLWTIEAISLLDL ISLKGKFSITALING DNQSIDISKPIRLME 825
GQTHAQADYLLALNS LKLLYKEYAGIGHKL KGTETYISRDMQFMS KTIQHNGVYYPASIK KVLRVGPWINTILDD 900
FKVSLESIGSLTQEL EYRGESLLCSLIFRN VWLYNQIALQLKNHA LCNNKLYLDILKVLK HLKTFFNLDNIDTAL 975
TLYMNLPMLFGGGDP NLLYRSFYRRTPDFL TEAIVHSVFILSYYT NHDLKDKLQDLSDDR LNKFLTCIITFDKNP 1050
NAEFVTLMRDPQALG SERQAKITSEINRLA VTEVLSTAPNKIFSK SAQHYTTTEIDLNDI MQNIEPTYPHGLRVV 1125
YESLPFYKAEKIVNL ISGTKSITNILEKTS AIDLTDIDRATEMMR KNITLLIRILPLDCN RDKREILSMENLSIT 1200
ELSKYVRERSWSLSN IVGVTSPSIMYTMDI KYTTSTISSGIIIEK YNVNSLTRGERGPTK PWVGSSTQEKKTMPV 1275
YNRQVLTKKQRDQID LLAKLDWVYASIDNK DEFMEELSIGTLGLT YEKAKKLFPQYLSVN YLHRLTVSSRPCEFP 1350
ASIPAYRTTNYHFDT SPINRILTEKYGDED IDIVFQNCISFGLSL MSVVEQFTNVCPNRI ILIPKLNEIHLMKPP 1425
IFTGDVDIHKLKQVI QKQHMFLPDKISLTQ YVELFLSNKTLKSGS HVNSNLILAHKISDY FHNTYILSTNLAGHW 1500
ILIIQLMKDSKGIFE KDWGEGYITDHMFIN LKVFFNAYKTYLLCF HKGYGKAKLECDMNT SDLLCVLELIDSSYW 1575
KSMSKVFLEQKVIKY ILSQDASLHRVKGCH SFKLWFLKRLNVAEF TVCPWVVNIDYHPTH MKAILTYIDLVRMGL 1650
INIDRIHIKNKHKFN DEFYTSNLFYINYNF SDNTHLLTKHIRIAN SELENNYNKLYHPTP ETLENILANPIKSND 1725
KKTLNDYCIGKNVDS IMLPLLSNKKLIKSS AMIRTNYSKQDLYNL FPMVVIDRIIDHSGN TAKSNQLYTTTSHQI 1800
SLVHNSTSLYCMLPW HHINRFNFVFSSTGC KISIEYILKDLKIKD PNCIAFIGEGAGNLL LRTVVELHPDIRYIY 1875
RSLKDCNDHSLPIEF LRLYNGHINIDYGEN LTIPATDATNNIHWS YLHIKFAEPISLFVC DAELSVTVNWSKIII 1950
EWSKHVRKCKYCSSV NKCMLIVKYHAQDDI DFKLDNITILKTYVC LGSKLKGSEVYLVLT IGPANIFPVFNVVQN 2025
AKLILSRTKNFIMPK KADKESIDANIKSLI PFLCYPITKKGINTA LSKLKSVVSGDILSY SIAGRNEVFSNKLIN 2100
HKHMNILKWFNHVLN FRSTELNYNHLYMVE STYPYLSELLNSLTT NELKKLIKITGSLLY NFHNE 2165
```

Charged Clusters (Amino Acids that are underlined were changed to alanines)

Mutations in cpts-248/404

Mutation in cpts530

FIG. 10

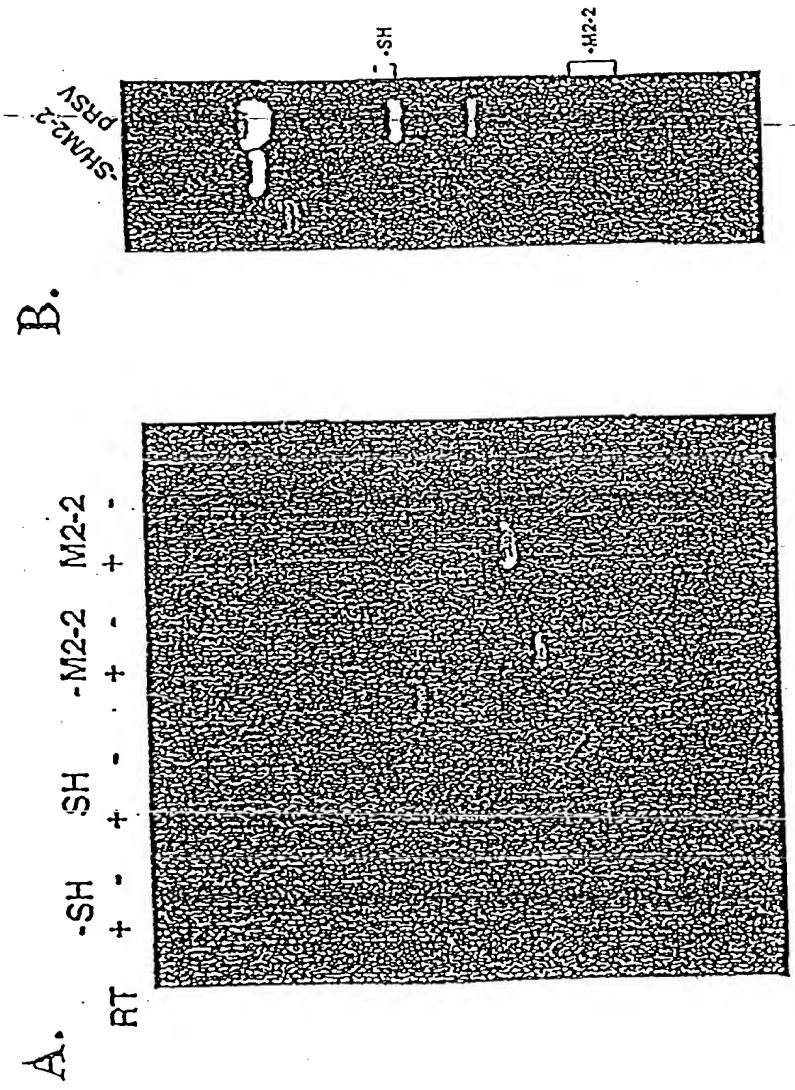
```
MDPIINGNSANVYLT DSYLKGVISFSECNA LGSYIFNGPYLKNDY TNLISRQNPLIEHMN LKKLNITQSLISKYH   75
KGEIKLEEPTYFQSL LMTYKSMTSSEQIAT TNLLKKIIRRAIEIS DVKVYAILNKLGLKE KDKIKSNNGQDEDNS  150
VITTIIKDDILSAVK DNQSHLKADKNHSTK QKDTIKTTLLKKLMC SMQHPPSWLIHWFNL YTKLNNILTQYRSNE  225
VKNHGFTLIDNQTLS GFQFILNQYGCIVYH KELKRITVTTYNQFL TWKDISLSRLNVCLI TWISNCLNTLNKSLG  300
LRCGFNNVILTQLFL YGDCILKLFHNEGFY IIKEVEGFIMSLILN ITEEDQFRKRFYNSM LNNITDAANKAQKNL  375
LSRVCHTLLDKTVSD NIINGRWIILLSKFL KLIKLAGDNNLNNLS ELYFLFRIFGHPMVD ERQAMDAVKINCNET  450
KFYLLSSLSMLRGAF IYRIIKGFVNNYNRW PTLRNAIVLPLRWLT YYKLNTYPSLLELTE RDLIVLSGLRFYREF  525
RLPKKVDLEMIINDK AISPPKNLIWTSFPR NYMPSHIQNYIEHEK LKFSESDKSRRVLEY YLRDNKFNECDLYNC  600
VVNQSYLNNPNHVVS LTGKERELSVGRMFA MQPGMFRQVQILAEK MIAENILQFFPESLT RYGDLELQKILELKA  675
GISNKSNRYNDNYNN YISKCSIITDLSKFN QAFRYETSCICSDVL DELHGVQSLFSWLHL TIPHVTIICTYRHAP  750
PYIGDHIVDLNNVDE QSGLYRYHMGGIEGW CQKLWTIEAISLLDL ISLKGKFSITALING DNQSIDISKPIRLME  825
GQTHAQADYLLALNS LKLLYKEYAGIGHKL KGTETYISRDMQFMS KTIQHNGVYYPASIK KVLRVGPWINTILDD  900
FKVSLESIGSLTQEL EYRGESLLCSLIFRN VWLYNQIALQLKNHA LCNNKLYLDILKVLK HLKTFFNLDNIDTAL  975
TLYMNLPMLFGGGDP NLLYRSFYRRTPDFL TEAIVHSVFILSYYT NHDLKDKLQDLSDDR LNKFLTCIITFDKNP 1050
NAEFVTLMRDPQALG SERQAKITSEINRLA VTEVLSTAPNKIFSK SAQHYTTTEIDLNDI MQNIEPTYPHGLRVV 1125
YESLPFYKAEKIVNL ISGTKSITNILEKTS AIDLTDIDRATEMMR KNITLLIRILPLDCN RDKREILSMENLSIT 1200
ELSKYVRERSWSLSN IVGVTSPSIMYTMDI KYTTSTISSGIIIEK YNVNSLTRGERGPTK PWVGSSTQEKKTMPV 1275
YNRQVLTKKQRDQID LLAKLDWVYASIDNK DEFMEELSIGTLGLT YEKAKKLFPQYLSVN YLHRLTVSSRPCEFP 1350
ASIPAYRTTNYHFDT SPINRILTEKYGDED IDIVFQNCISFGLSL MSVVEQFTNVCPNRI ILIPKLNEIHLMKPP 1425
IFTGDVDIHKLKQVI QKQHMFLPDKISLTQ YVELFLSNKTLKSGS HVNSNLILAHKISDY FHNTYILSTNLAGHW 1500
ILIIQLMKDSKGIFE KDWGEGYITDHMFIN LKVFFNAYKTYLLCF HKGYGKAKLECDMNT SDLLCVLELIDSSYW 1575
KSMSKVFLEQKVIKY ILSQDASLHRVKGCH SFKLWFLKRLNVAEF TVCPWVVNIDYHPTH MKAILTYIDLVRMGL 1650
INIDRIHIKNKHKFN DEFYTSNLFYINYNF SDNTHLLTKHIRIAN SELENNYNKLYHPTP ETLENILANPIKSND 1725
KKTLNDYCIGKNVDS IMLPLLSNKKLIKSS AMIRTNYSKQDLYNL FPMVVIDRIIDHSGN TAKSNQLYTTTSHQI 1800
SLVHNSTSLYCMLPW HHINRFNFVFSSTGC KISIEYILKDLKIKD PNCIAFIGEGAGNLL LRTVVELHPDIRYIY 1875
RSLKDCNDHSLPIEF LRLYNGHINIDYGEN LTIPATDATNNIHWS YLHIKFAEPISLFVC DAELSVTVNWSKIII 1950
EWSKHVRKCKYCSSV NKCMLIVKYHAQDDI DFKLDNITILKTYVC LGSKLKGSEVYLVLT IGPANIFPVFNVVQN 2025
AKLILSRTKNFIMPK KADKESIDANIKSLI PFLCYPITKKGINTA LSKLKSVVSGDILSY SIAGRNEVFSNKLIN 2100
HKHMNILKWFNHVLN FRSTELNYNHLYMVE STYPYLSELLNSLTT NELKKLIKITGSLLY NFHNE           2165
```

C — Cysteine residues

C — Cysteine residues that were changed to valine or aspartic acid

C — Cysteine residue deleted

FIG. 11



FIGS. 12A-B

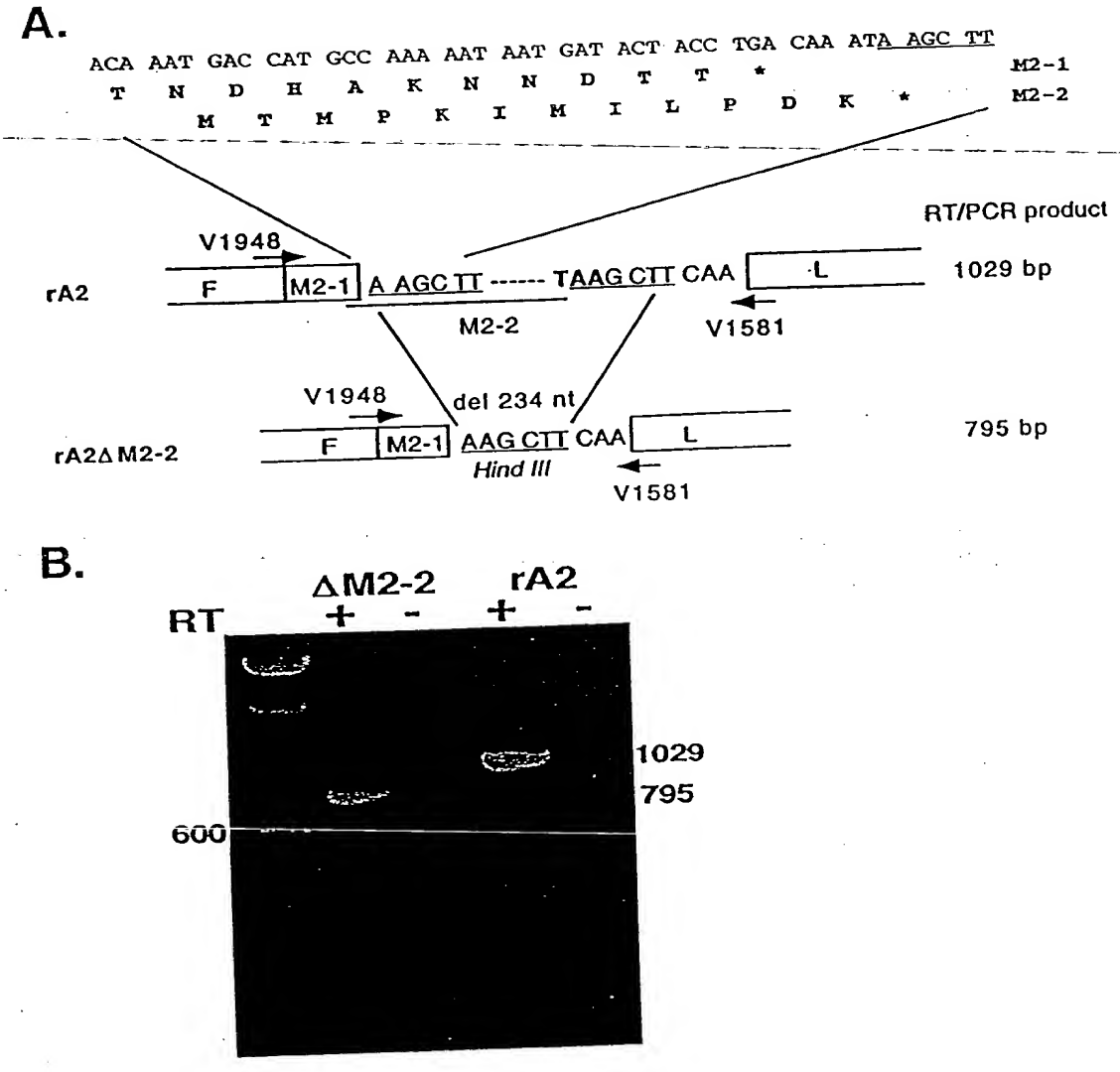

A.
ACA AAT GAC CAT GCC AAA AAT AAT GAT ACT ACC TGA CAA ATA AGC TT
T N D H A K N N D T T *
M T M P K I M I L P D K *
M2-1
M2-2
RT/PCR product
V1948
rA2
F
M2-1
A AGC TT ------ TAAG CTT CAA
L
1029 bp
M2-2
V1581
V1948
del 234 nt
rA2ΔM2-2
F
M2-1
AAG CTT CAA
L
795 bp
Hind III
V1581
B.
ΔM2-2
rA2
RT
+ - + -
1029
795
600

FIG. 13

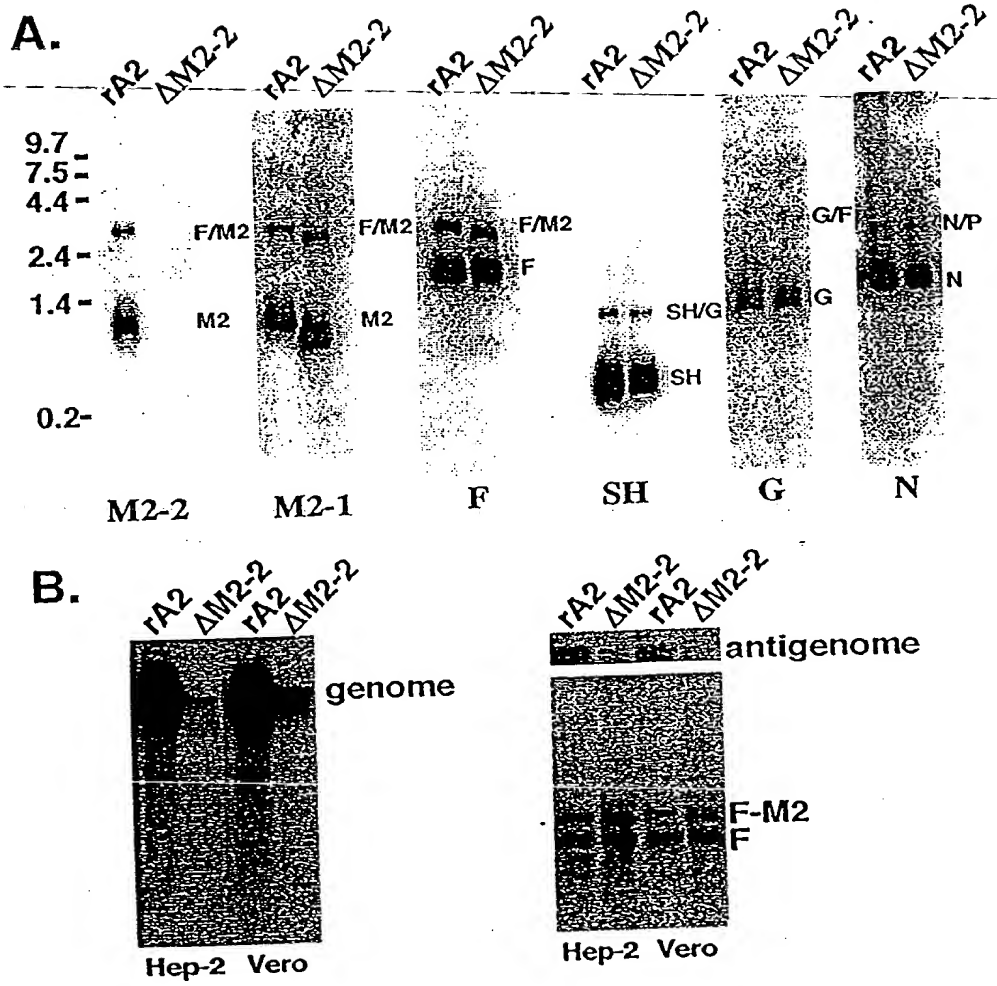

A.
rA2
ΔM2-2
9.7
7.5
4.4
2.4
1.4
0.2
F/M2
M2
F
SH/G
SH
G/F
G
N/P
N
M2-2
M2-1
F
SH
G
N
B.
genome
antigenome
F-M2
F
Hep-2
Vero

FIG. 14

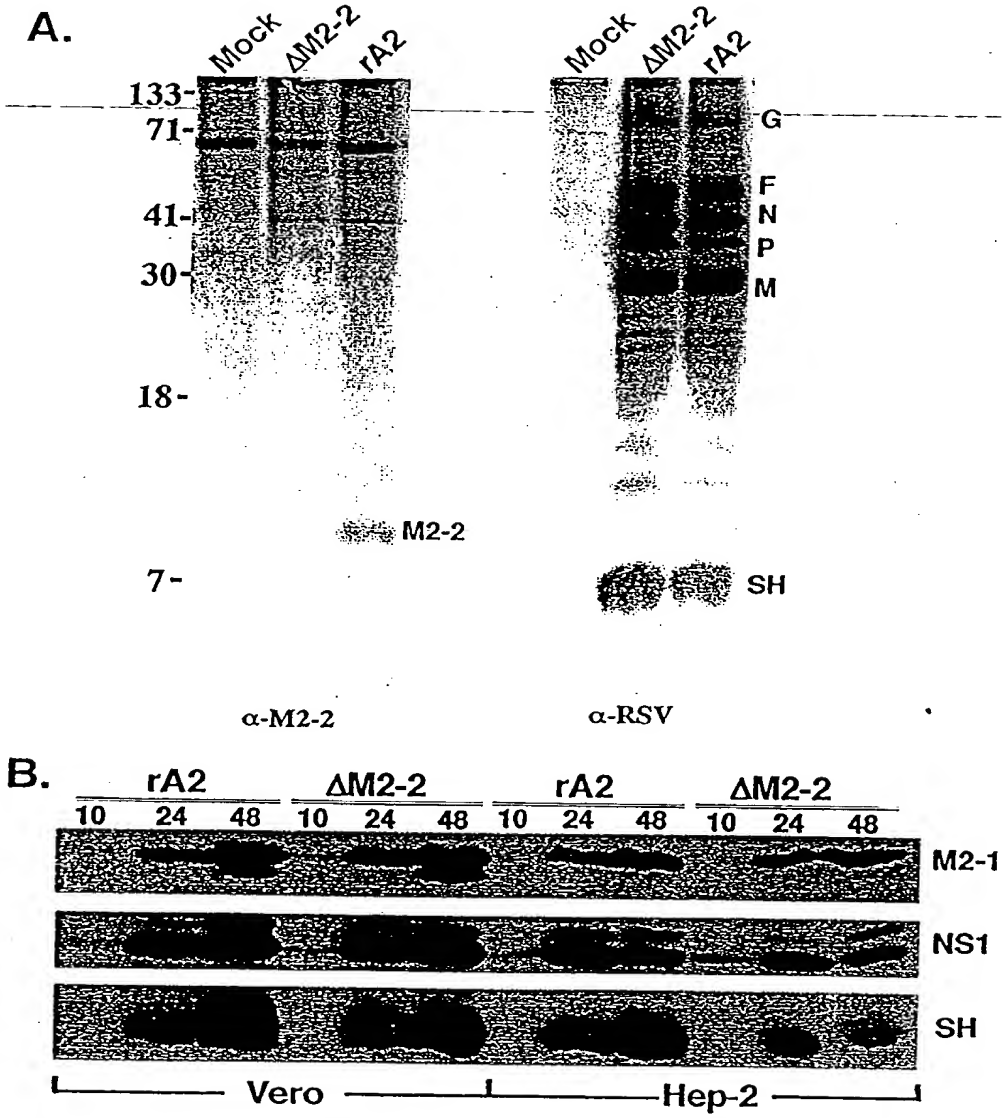

A.
Mock
ΔM2-2
rA2
Mock
ΔM2-2
rA2
133-
71-
41-
30-
18-
7-
G
F
N
P
M
M2-2
SH
α-M2-2
α-RSV
B.
rA2
ΔM2-2
rA2
ΔM2-2
10 24 48 10 24 48 10 24 48 10 24 48
M2-1
NS1
SH
Vero
Hep-2

FIG. 15

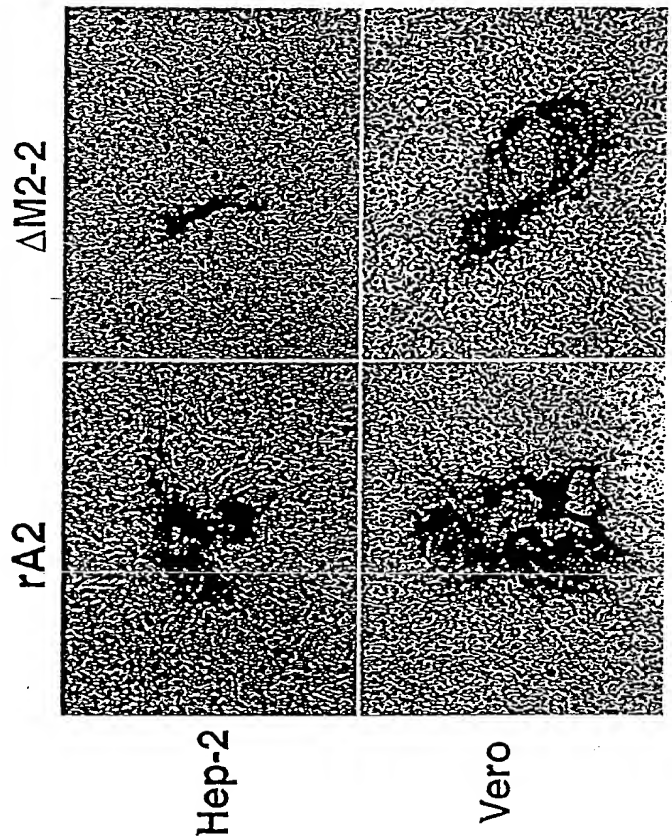


FIG. 16

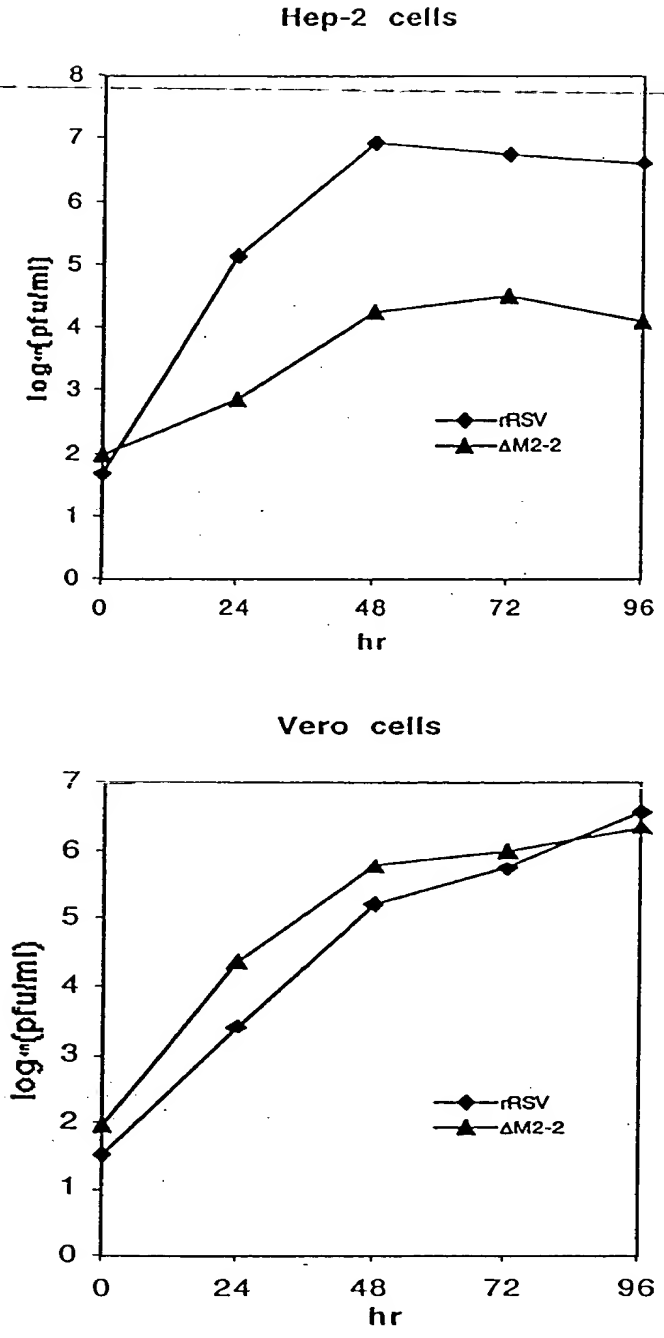

Hep-2 cells
log10(pfu/ml)
hr
rRSV
ΔM2-2
Vero cells
log10(pfu/ml)
hr
rRSV
ΔM2-2

FIG. 17

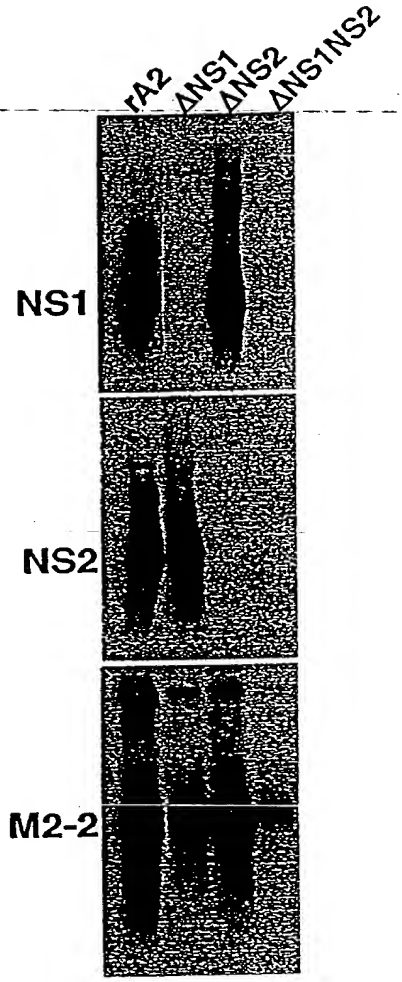


FIG. 18

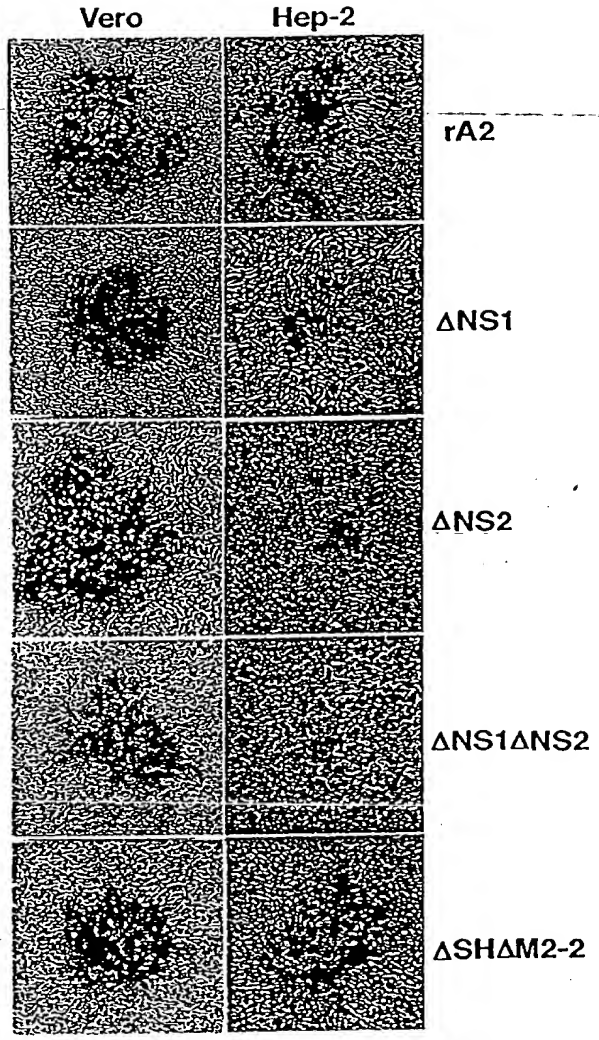


FIG. 19

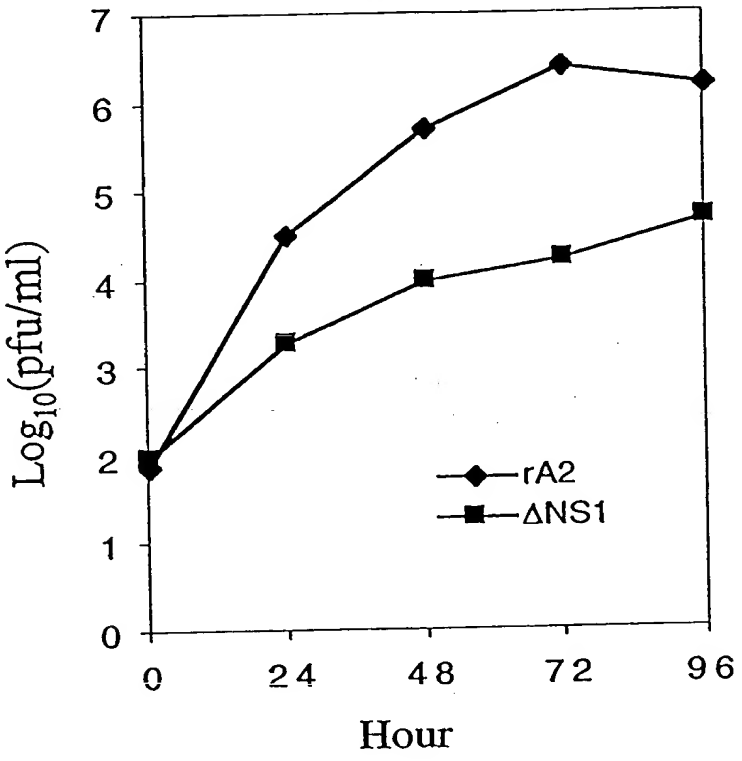


FIG. 20

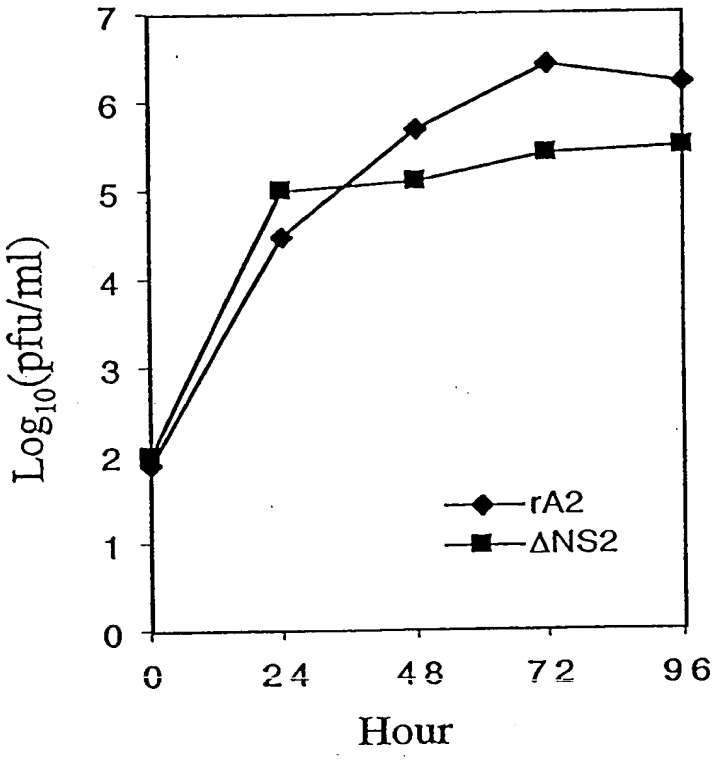


FIG. 21

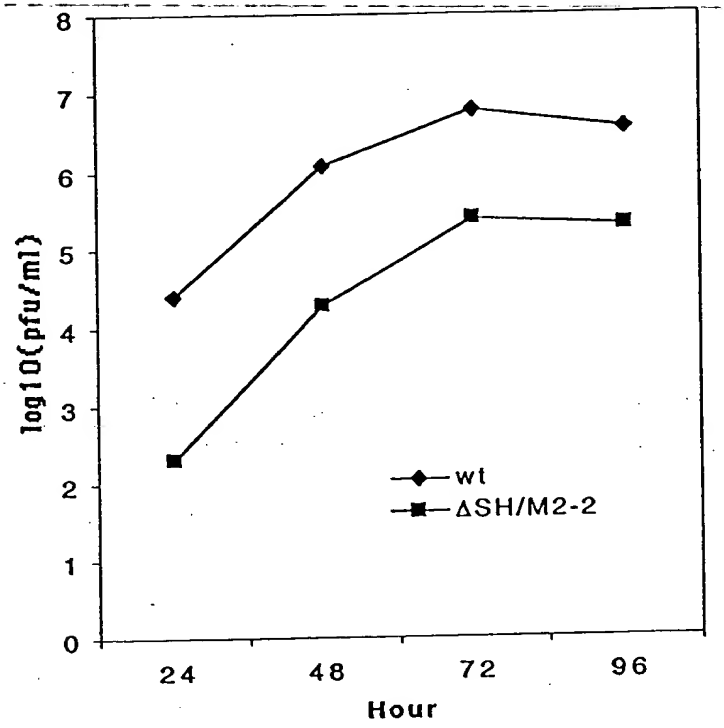


FIG. 22

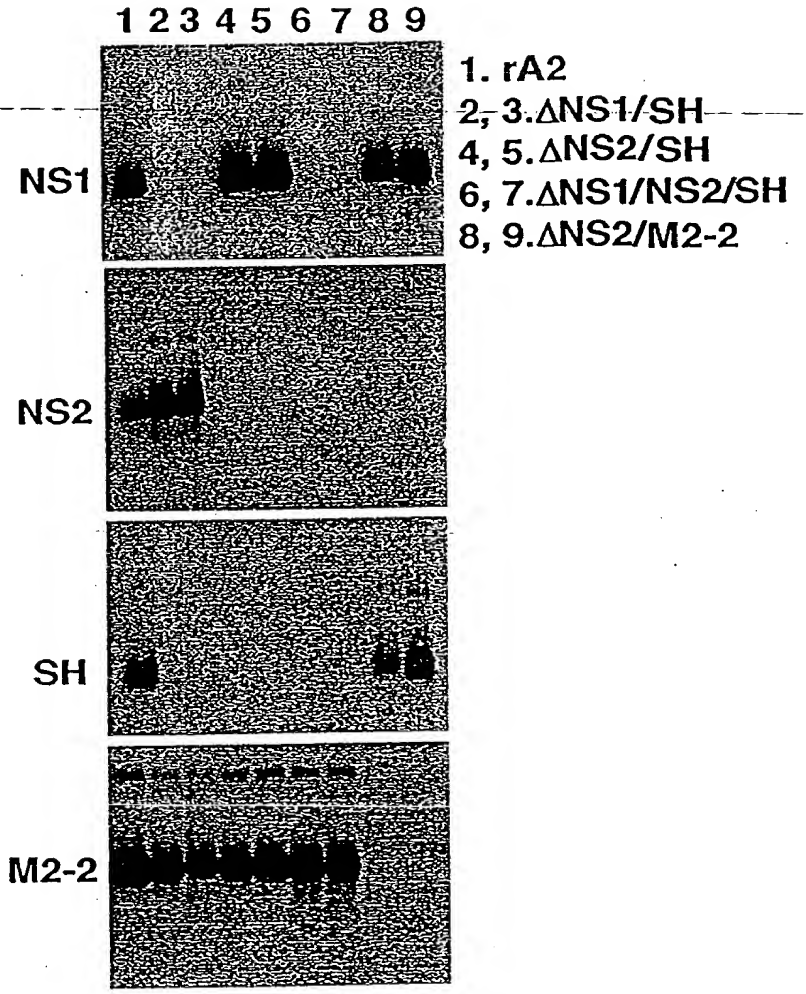

1 2 3 4 5 6 7 8 9
NS1
NS2
SH
M2-2
1. rA2
2, 3.ΔNS1/SH
4, 5.ΔNS2/SH
6, 7.ΔNS1/NS2/SH
8, 9.ΔNS2/M2-2

FIG. 23

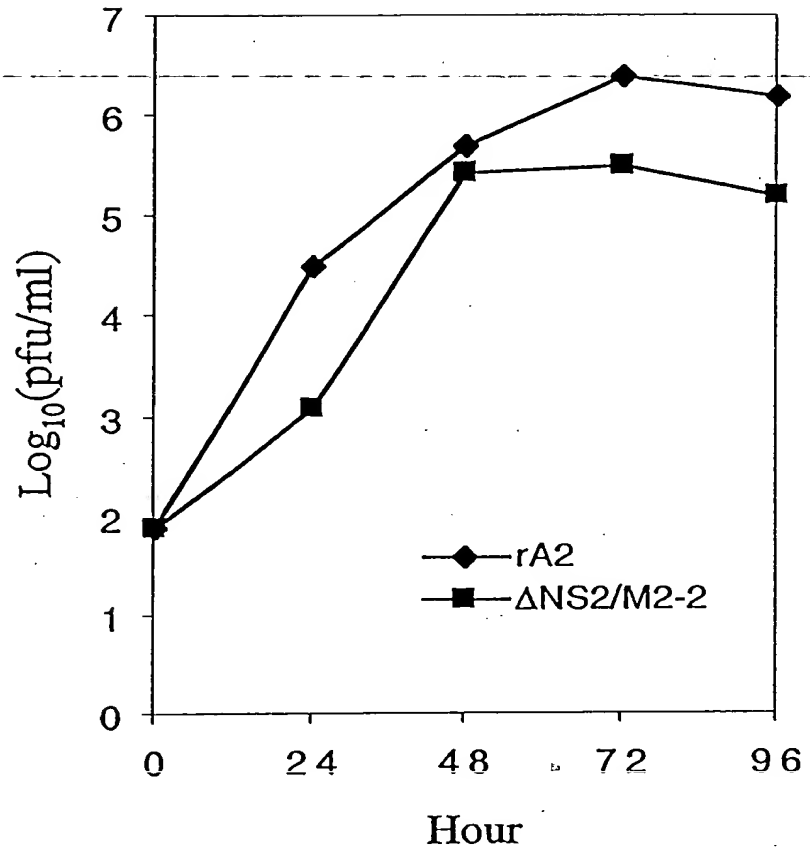


FIG. 24

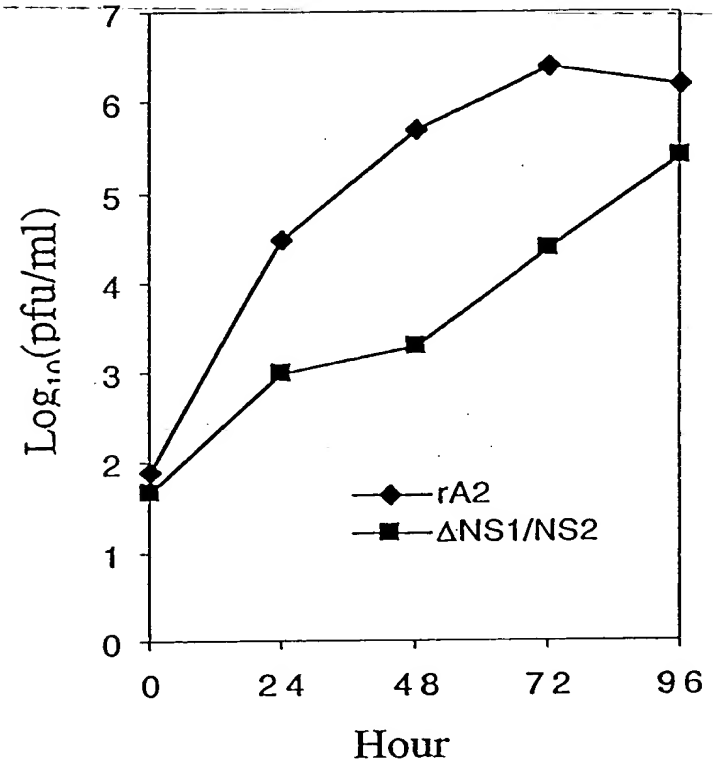


FIG. 25

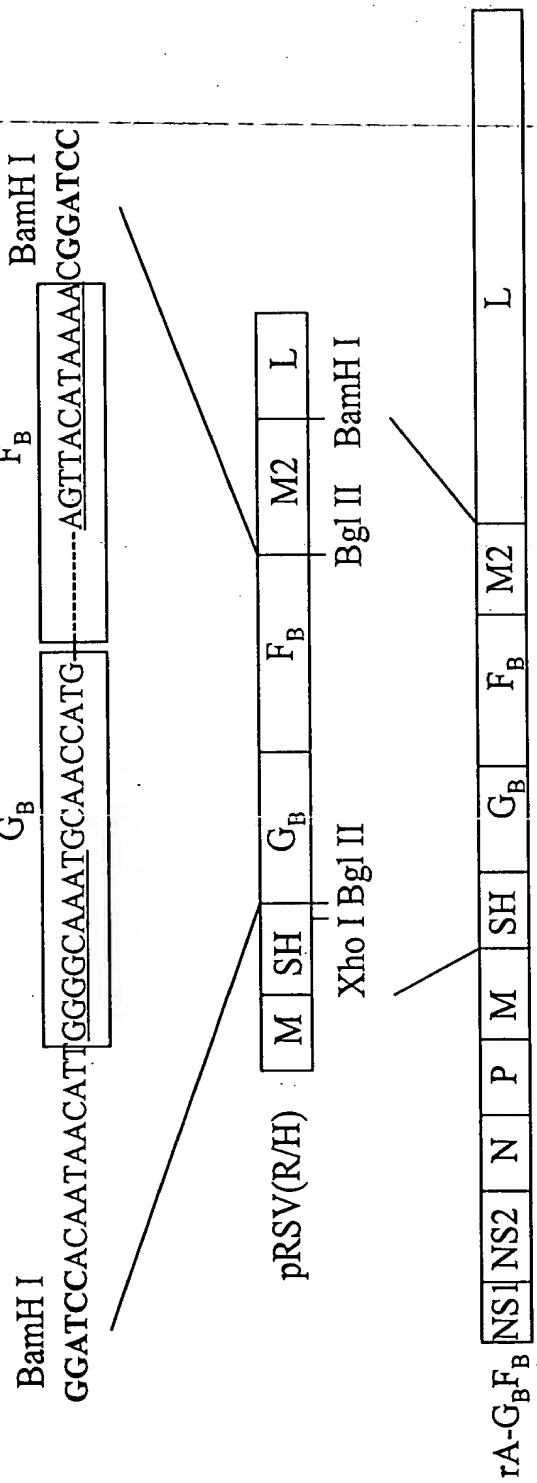

BamH I
GGATCCACAATAACATGGGGCAAATGCAACCATG
G_B
F_B
BamH I
CGGATCC
AGTTACATAAA
pRSV(R/H)
M
SH
G_B
F_B
M2
L
Xho I Bgl II
Bgl II BamH I
rA-$G_B F_B$
NS1
NS2
N
P
M
SH
G_B
F_B
M2
L

FIG. 26

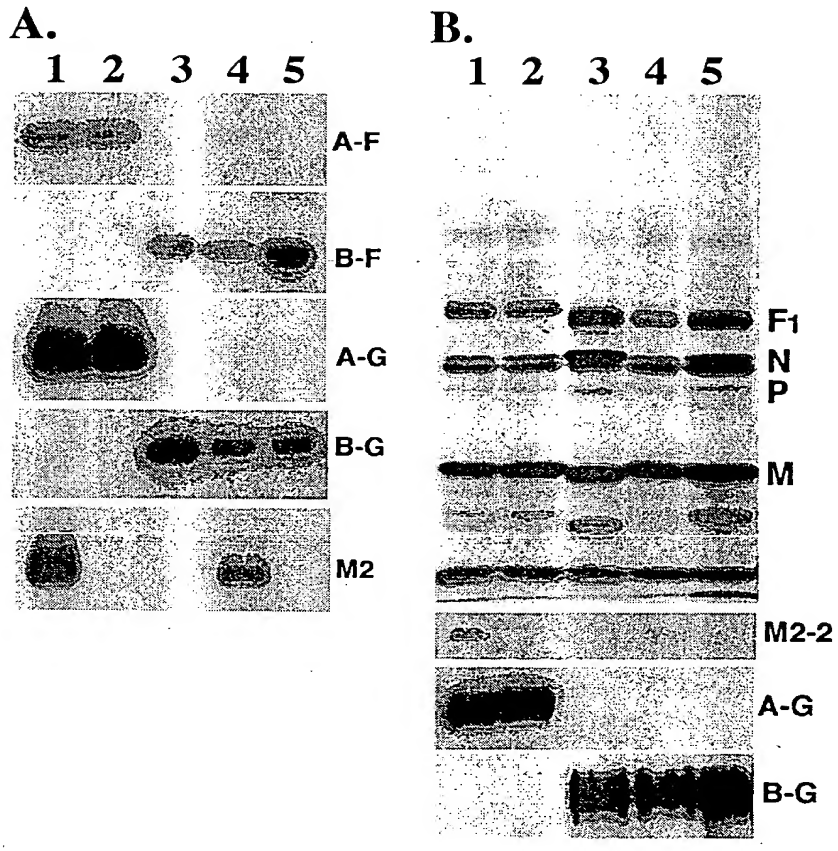


FIG. 27

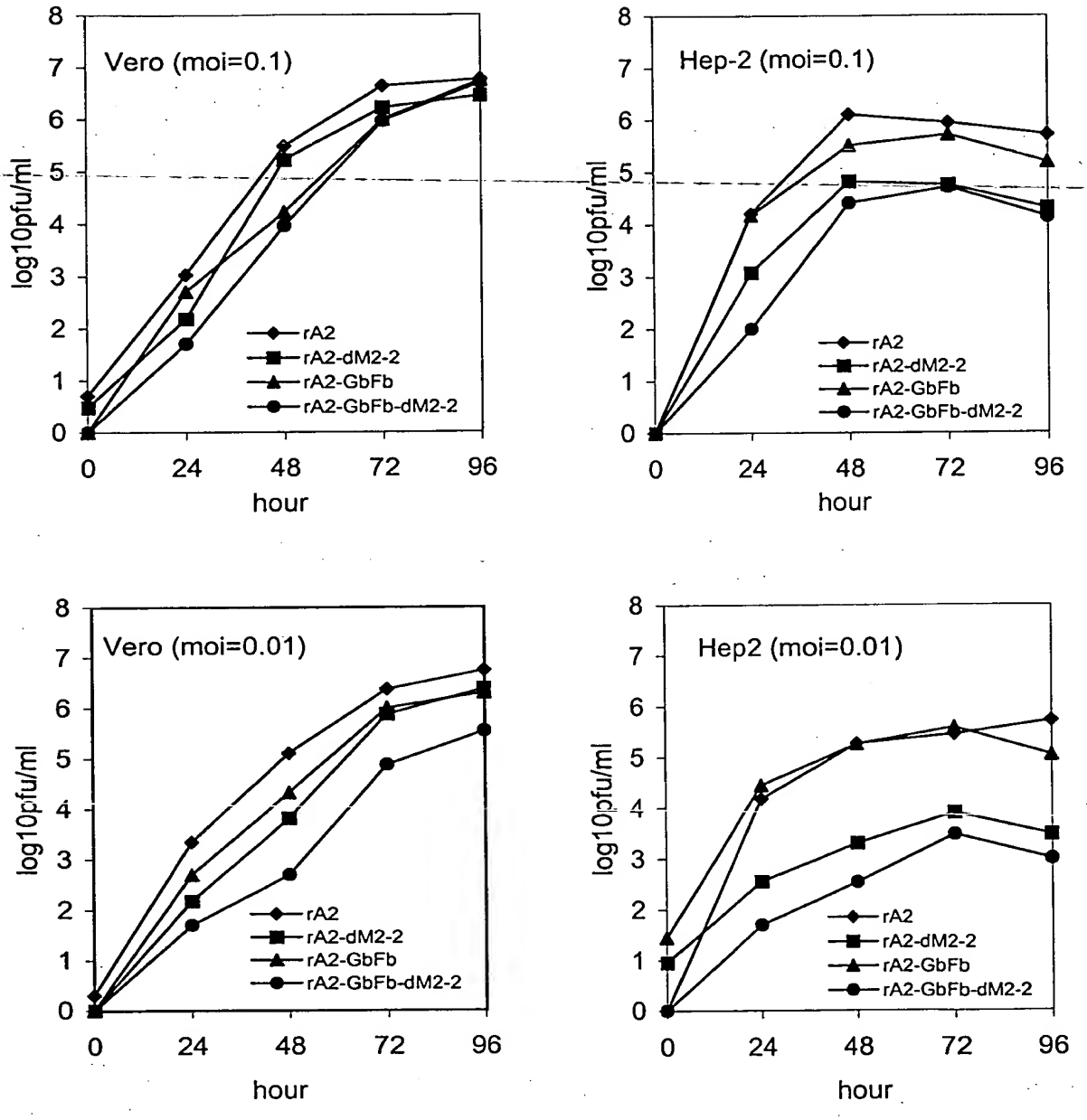

Vero (moi=0.1)
Hep-2 (moi=0.1)
Vero (moi=0.01)
Hep2 (moi=0.01)
log10pfu/ml
hour
0
24
48
72
96
rA2
rA2-dM2-2
rA2-GbFb
rA2-GbFb-dM2-2

FIG. 28